PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2402PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/AU2003/000898	International Filing Date *(day/month/year)* 11 July 2003	Priority Date *(day/month/year)* 12 July 2002

International Patent Classification (IPC) or national classification and IPC

Int. Cl.[7] C07K 14/47, 14/475, 14/65; C07H 21/04; A61K 38/17, 38/18, 38/30; A61P 35/00

Applicant

THE UNIVERSITY OF ADELAIDE et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheet(s).

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of the report
19 January 2004	26 February 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer G THOMPSON Telephone No. (02) 6283 2240

INTERNATIONAL PRE[redacted]NARY EXAMINATION REPORT

[redacted]ernational application No.
PCT/AU2003/000898

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1-25** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☒ the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **26-30** received on **19 January 2004** with the letter of **19 January 2004**

☒ the drawings, pages **1/6-6/6** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☒ the sequence listing part of the description:
pages **1/4-4/4** as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims **1-39**	**YES**
	Claims	**NO**
Inventive step (IS)	Claims **1-39**	**YES**
	Claims	**NO**
Industrial applicability (IA)	Claims **1-39**	**YES**
	Claims	**NO**

2. Citations and explanations (Rule 70.7)

NOVELTY (N) AND INVENTIVE STEP (IS) Claims 1-39 ACKNOWLEDGED

Journal of biotechnology, Vol. 61, 1998, Lucic, M. et al, "Secretion in Escherichia coli and phage display of recombinant insulin-like growth factor binding protein-2" pp. 95-108

Journal of Molecular Endocrinology, Vol. 23, 1999, Bramani S. et al., "Amino acids within the extracellular matrix (ECM) binding region (201-218) of rat insulin-like growth factor binding protein (IGFBP)-5 are important determinants in binding IGF-I", pp. 117-123

Lucic et al discloses that "mutants of IGFBP-2 with a higher affinity for IGF-II than [the] wild type might be difficult to elute with acid or by competition with IGF-II (p. 105 right col. l.24-27)." But there is no disclosure of inhibited release of IGF from an altered IGFBP-2 when an extracellular matrix (ECM) or protease is contacted.

Bramani et al indicates that the analogous IGFBP-5 has reduced IGF-I affinity when the former is ECM bound (end of the abstract). Contrary to this tendency, the instant invention reports an (altered) IGFBP that inhibits the release of IGF-I and IGF-II when contacting the ECM (p.3 l.15-31).

The said claims are therefore novel and inventive.

INDUSTRIAL APPLICABILITY (IA) Claims 1-39

While no unified criteria exist for determining what belongs in this category, there is nothing evident in the claims that would deprive them of affirmation in this category.

CLAIMS

1. An altered IGFBP-2 molecule able to effect binding of IGF-I or IGF-II with high affinity characterised in having an inhibited release of IGF on contact with extracellular matrix or exposure to a protease.

2. An altered IGFBP molecule of claim 1 wherein the IGFBP-2 molecule has an alteration in one or more amino acids of a first ECM binding sequence which spans amino acids 179-184 and comprises the sequence PKKLRP [SEQ ID No 1].

3. An altered IGFBP molecule as in either claim 1 or 2 wherein the altered IGFBP-2 molecule has an alteration in one or more amino acids of a second ECM binding sequence which spans amino acids 227-244 and comprises the sequence KHGLYNLKQCKMSLNGQR [SEQ ID No 2].

4. An altered IGFBP molecule as in claim 2 wherein the alteration in the first ECM binding sequence is selected from the group consisting of SEQ ID No 9, SEQ ID No 10, SEQ ID No 11, SEQ ID No 12 and SEQ ID No 13.

5. An altered IGFBP molecule as in claim 3 wherein the alteration in the second ECM binding sequence is selected from the group consisting of SEQ ID No 14, SEQ ID No 15, SEQ ID No 16, SEQ ID No 17 and SEQ ID No 18.

6. An altered IGFBP molecule as in claim 1 wherein the amino acid sequence is alterated as one or both of K180A and K181A

7. An altered IGFBP molecule as in either claim 2 or 3 wherein the IGFBP-2 molecule has additionally an alteration of its amino acid sequence that enhances resistance to proteolysis by one or more proteases.

8. An altered IGFBP molecule as in claim 7 wherein the IGFBP-2 molecule has an amino acid sequence altered in the linker domain to provide resistance to said one or more proteases.

9. An altered IGFBP molecule as claim 7 having one or more amino acids deleted within the linker domain

10. An altered IGFBP molecule as claim 7 wherein substantially all of the linker domain is deleted, but said altered molecule still retains amino acids from about 180 through to 191.

11. An altered IGFBP molecule as in claim 7 wherein the IGFBP-2 molecule has a deletion of amino acids 114 through to 170.

12. An altered IGFBP molecule as in claim 1 wherein the IGFBP-2 molecule additionally has an amino acid sequence altered to enhances resistance to proteolysis by one or more proteases.

13. An altered IGFBP molecule as in claim 1 wherein the IGFBP-2 molecule has an amino acid sequence alteration in the linker domain to provide resistance to said one or more proteases.

14. An altered IGFBP molecule as claim 1 having one or more amino acids deleted within the linker domain

15. An altered IGFBP molecule as claim 1 wherein substantially all of the linker domain is deleted, but said altered molecule still retains amino acids from about 180 through to 191.

16. An altered IGFBP molecule as in claim 1 wherein the IGFBP-2 molecule has a deletion of amino acids 114 through to 170.

17. An altered IGFBP molecule as in claim 13 wherein the amino acid sequence is alterated at one or both positions as follows K180A and K181A.

18. A nucleic acid encoding an altered IGFBP-2 molecule said altered IGFBP molecule able to effect binding of IGF-I or IGF-II with high affinity characterised in having an inhibited release of IGF on contact with extracellular matrix or exposure to a protease.

19. A nucleic acid encoding an altered IGFBP molecule as claim 18 wherein the IGFBP-2 molecule has an alteration in one or more amino acids of a first ECM binding sequence which spans amino acids 179-184 and comprises the sequence PKKLRP [SEQ ID No 1]

20. A nucleic acid encoding an altered IGFBP molecule as in either claim 18 or 19 wherein the altered IGFBP-2 molecule has an alteration in one or more amino acids of a second ECM binding sequence which spans amino acids 227-244 and comprises the sequence KHGLYNLKQCKMSLNGQR [SEQ ID No 2].

21. A nucleic acid encoding an altered IGFBP molecule as in claim 19 wherein the alteration in the first ECM binding sequence is selected from the group consisting of SEQ ID No 9, SEQ ID No 10, SEQ ID No 11, SEQ ID No 12 and SEQ ID No 13.

22. A nucleic acid encoding an altered IGFBP molecule as in claim 20 wherein the alteration in the second ECM binding sequence is selected from the group consisting of SEQ ID No 14, SEQ ID No 15, SEQ ID No 16, SEQ ID No 17 and SEQ ID No 18.

23. A nucleic acid encoding an altered IGFBP molecule as in claim 18 wherein the amino acid sequence is altered in one or both of K180A and K181A

24. A nucleic acid encoding an altered IGFBP molecule as in either claim 19 or 20 wherein the IGFBP-2 molecule has additionally an alteration of its amino acid sequence that enhances resistance to proteolysis by one or more proteases.

25. A nucleic acid encoding an altered IGFBP molecule as in claim 24 wherein the IGFBP-2 molecule has an amino acid sequence alteration in the linker domain to provide resistance to said one or more proteases.

26. A nucleic acid encoding an altered IGFBP molecule as claim 24 having one or more amino acids deleted within the linker domain

27. A nucleic acid encoding an altered IGFBP molecule as claim 24 wherein substantially all of the linker domain is deleted, but said altered molecule still retains amino acids from about 180 through to 191.

28. A nucleic acid encoding an altered IGFBP molecule as in claim 24 wherein the IGFBP-2 molecule has a deletion of amino acids 114 through to 170.

29. A nucleic acid encoding an altered IGFBP molecule as in claim 24 wherein the IGFBP-2 molecule additionally has an amino acid sequence altered to enhance resistance to proteolysis by one or more proteases.

30. A nucleic acid encoding an altered IGFBP molecule as in claim 18 wherein the IGFBP-2 molecule has an amino acid sequence altered in the linker domain to provide resistance to said one or more proteases.

31. A nucleic acid encoding an altered IGFBP molecule as claim 18 having one or more amino acids deleted within the linker domain

32. A nucleic acid encoding an altered IGFBP molecule as claim 18 wherein substantially all of the linker domain is deleted, but said altered molecule still retains amino acids from about 180 through to 191.

33. A nucleic acid encoding an altered IGFBP molecule as in claim 18 wherein the IGFBP-2 molecule has a deletion of amino acids 114 through to 170.

34. A nucleic acid encoding an altered IGFBP molecule as in claim 29 wherein the amino acid sequence is alterated at one or both positions as follows K180A and K181A

35. A nucleic acid encoding an altered IGFBP molecule as in any one of claims 19 to 36 wherein the nucleic acid is a vector, the vector having nucleic acid operably linked with a control sequence including a promoter for transcription leading to expression of the altered IGFBP.

36. A host cell carrying a nucleic acid as in any one of claims 18 to 35.

37. A method of reducing IGF mediated proliferation of a population of cancerous cells, the method including the step of contacting the population of cells with an altered IGFBP as in any one of claims 1 to 17.

38. The method of reducing IGF mediated proliferation of a population of cancerous cells as in claim 39 wherein the cancerous cells are selected from the group consisting of prostate, colon and breast cancer cells.

39. The method of reducing IGF mediated proliferation of a population of cancerous cells as in claim 37 wherein the cancerous cells are colon cancer cells.